Empirical Valence Bond Simulations of Glutathione Peroxidase

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SUMMARY

Selenoproteins are broadly divided into three families such as Glutathione peroxidases (GPXs), Thioredoxin reductases (TRs) and Iodothyronine deiodinases (DIOs).[1],[7] Different isoforms of Glutathione Peroxidase (GPX) have been labeled as potential targets to control or cause the oxidative stress during cancer evolution.[4],[5] Mammalian GPX1, GPX2, GPX3, and GPX4 are selenium containing enzymes, GPX6 is a selenoprotein in humans with cysteine containing homologous in rodents. GPX5, GPX7 and GPX8 contain Cysteine. [6],[7] The relationships between the mechanism and the structure is not completely known, which has led us to investigate the sequence-structure-function relationships. Experimental and computational results through Empirical Valence Bond Simulations in previous work show that catalytic activity of several reconstructed ancestral structures of GPX6 recover their peroxidase activity when the active site is mutated from Cys to Sec keeping the binding of glutathione in all cases. The current state is that we have understanding of the concerted mechanism of the reaction, which has given us the possibility to set up the simulations in the mouse and human wild type enzymes. Our goal is to study the epistasis linked to the accumulation of amino acid variants that may explain the presence and absence of peroxidase activity in current isoforms beyond the need for the Sec in the active site.

CENTRAL QUESTION

GPX6 appears to lose its peroxidase activity when Cys is replaced by Sec in the active site. Does this loss happen only due to a single mutation or accumulation of mutations have a significant role to play in this process?

What Is Glutathione Peroxidase

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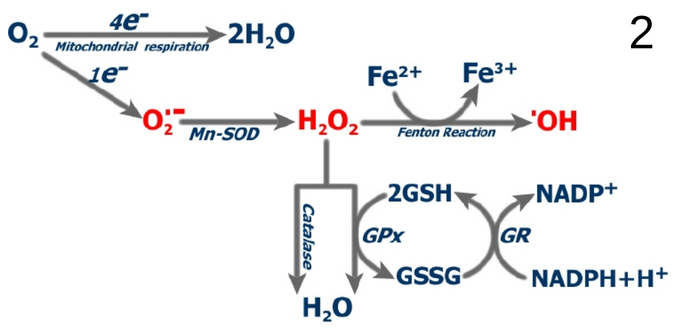

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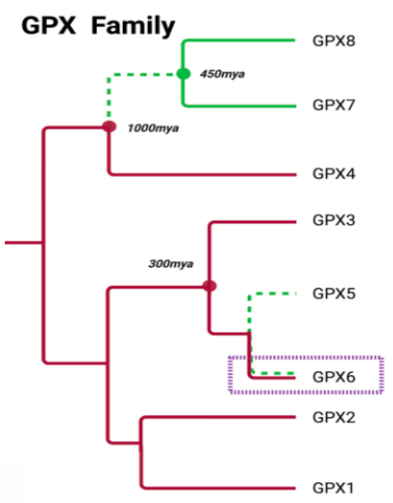


Fig 1. Generation and scavenging of reactive oxygen species (ROS) [8]

Fig 2. The phylogeny of the GPX family in Eukaryotes

HYPOTHESIS

3

There appears to exist an evolutionary shift from Sec to Cys sequences in the glutathione peroxidase family. Due to this shift, the peroxidase activity is lost in GPX6 when Se is replaced with S. We hypothesize that the loss may be in addition, correlated with the accumulation of mutations

OBJECTIVES

1.To explore the mode of interaction between GPX6 and the glutathione co-factor in ancestral to modern GPX6.

2. To understand enzymatic mechanism of GPX6.

3. To understand the effect of the mutations on the peroxidase activity of GPX6.

4. To discriminate the possible evolutionary pathways that link the ancestral protein with modern one.

STEP 1

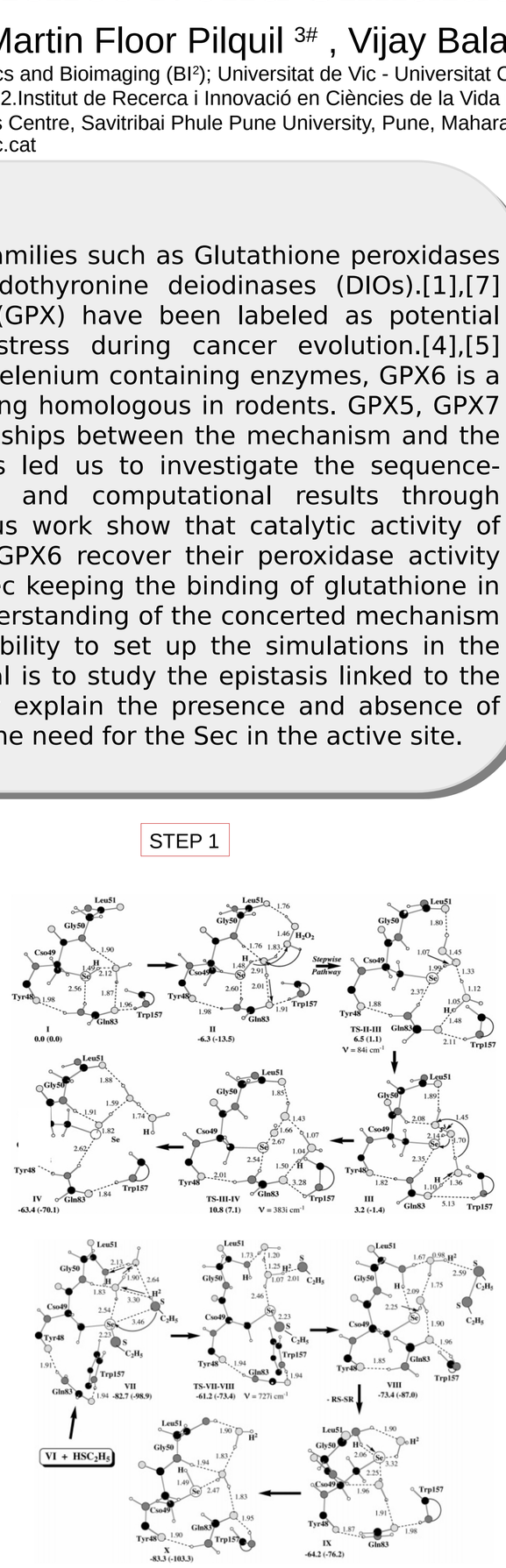
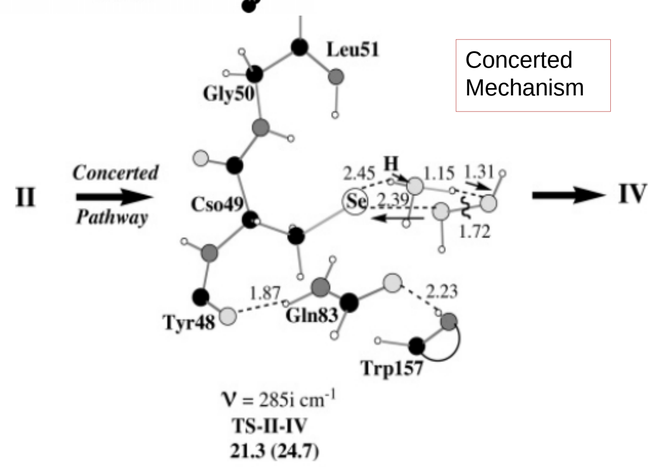


Fig 3. Stepwise and concerted mechanism of Bovine GPX1 [2]

STEP 2

STEP 3

Concerted Mechanism



RESULTS

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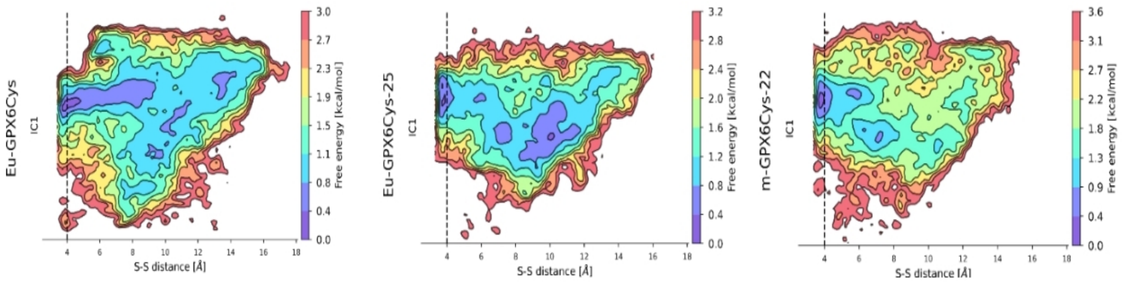
Active site - Cys/Sec Catalytic Tetrad – Cys/Sec, Trp (Tryptophan), Asn (Asparagin) and Gln/Ser (Glutamine/Serine)

Fig 4. The image shows structural homology of all isoforms of GPX in human and mouse

5a



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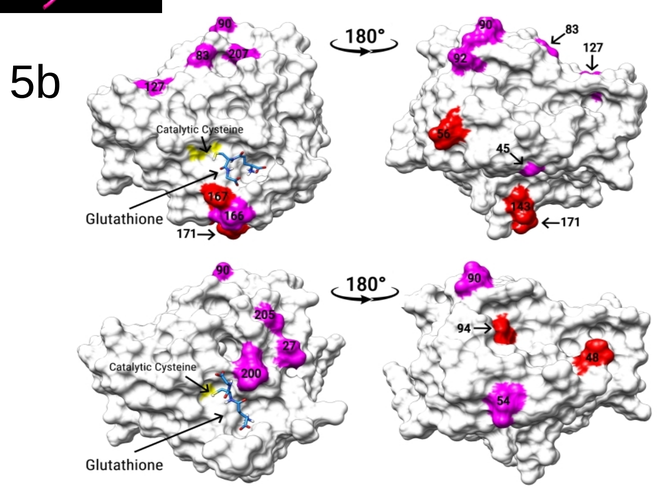

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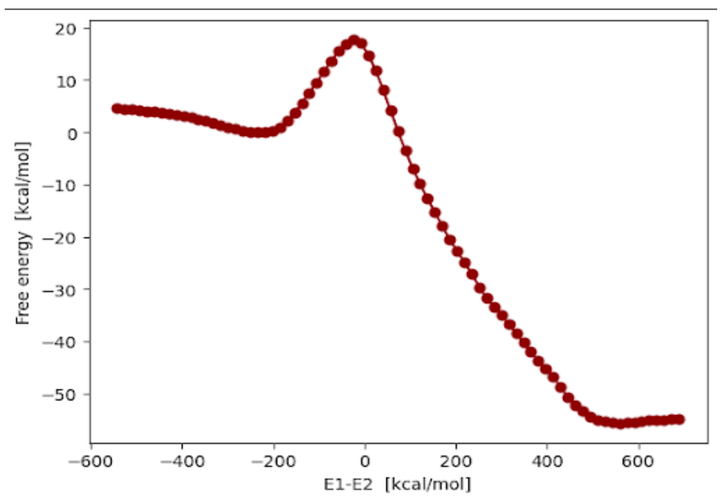

Previous computational analysis suggests that the binding of GSH and overall structures of the enzymes have not been adversely affected by the involvement of Cys. The conservation of GPX6-glutathione interactions, despite the loss of peroxidase activity may suggest additional functional roles of GPX6 still to be described. [3]

Fig 5a. Free energy profiles for the docking of the glutathione dimer to ancestral and modern GPX6 Cys proteins. [3]
Fig 5b - Convergence patterns from Eu-GPX6Cys to Eu-GPX6Cys+25 (top) and from Eu-GPX6Cys+25 to m-GPX6Cys+22 (Mouse-GPX6) (bottom).
The catalytic cysteine (yellow) is shown with the glutathione best binding energy conformation. [3]
Fig 5c. Free energy plot obtained from the EVB simulations of concerted mechanism of Wild type Mammalian Sec-containing GPX6 protein.

FURTHER WORK

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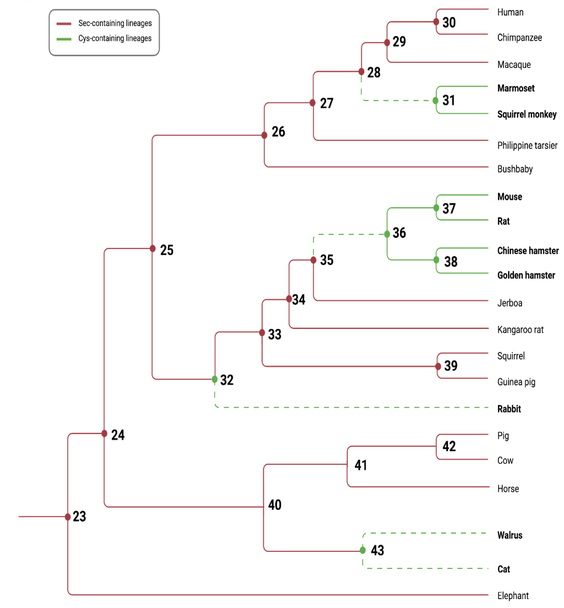

The goal is running free energy calculations on a collection of variants of GPX6 representing the corresponding nodes in the phylogenetic tree (Fig 6) obtained in our previous work. The EVB simulations will be carried out by comparing all 43 nodes in the phylogenetic tree with two reference systems - the modern (Cys containing) Mouse and (Sec-containing) Human GPX6. Expecting to identify accompanying mutations and their ability to maintain or lose peroxidase activity beyond the replacement of Sec by Cys.

METHODOLOGY

1.Alphafold2 for protein structure reconstruction from ancestral sequences.
2.Molecular dynamics simulations (OpenMM, AMBER ff) to explore the conformational landscapes of GPX6 and its complexes with GSSG
3.Protein-ligand binding energy landscape explorations was done using the PELE software (Protein Energy Landscape Exploration)
4.Q6 program was used to run Empirical Valence Bond Simulations

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